Propagation and Culture of Freshwater Mussels In Vitro

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In Vitro Propagation History

- Early 1900s - unionid propagation began in the US.
  - Ellis and Ellis (1926) – developed first transformation medium for artificial culture
- Fish physiology was a prerequisite for the development of complete artificial culture media
  - Isom and Hudson (1982) – modified Ellis and Ellis' culture medium to develop several media that were successful in transforming glochidia.

Artificial Culture Techniques - Components

- Ionic Balance
  - Unionid Ringers, Earle's or Hank's
- Whole Protein (sera or plasma)
  - Fish plasma, rabbit serum, horse serum
- Serum Replacements(Isom and Hudson 1982)
  - TCH, TCM only with sera
- Antibiotics/Antimycotics (Isom and Hudson 1982)
  - Carbenicillin, gentamicin sulfate and rifampin ; amphotericin B
- Other Medium Components
  - Glucose, phenol red, cod liver oil

jury is still out on this…

Advantages and Disadvantages: In Vitro

Advantages

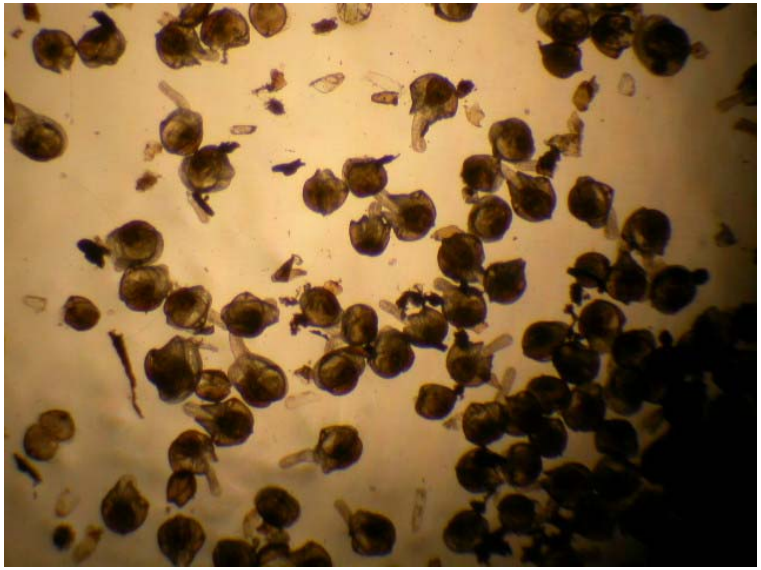
- ability to obtain more juveniles per unit effort.
- when host fish are unknown, this technique can produce juveniles in many cases.
- for monitoring efforts, *in vitro* juveniles may be more sensitive than *in vivo*-cultured juveniles.
- Able to visually see growth and development taking place, which provides an of the requirements as transformation occurs.

Disadvantages

- costs can be considerably higher
- microbial and fungal infestation can eliminate transforming glochidia if not closely monitored.
- for monitoring efforts, *in vitro* juveniles may be more sensitive than *in vivo*-cultured juveniles.

Are *in vitro* Cultures Viable?

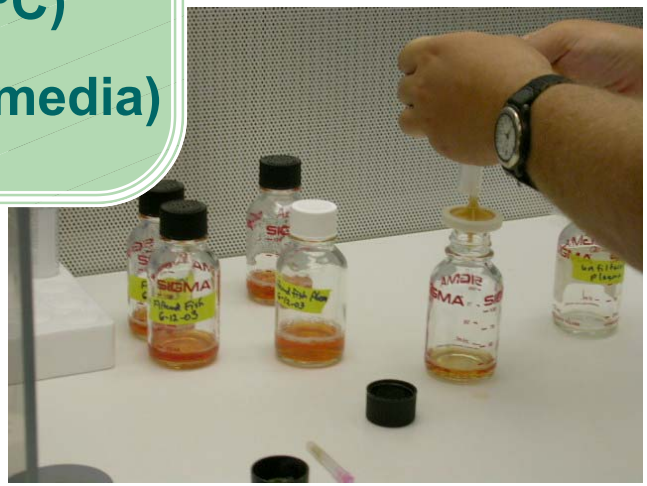
- Very often, artificial culture techniques can produce significantly more juveniles than host fish techniques. The utilization of artificial culture should not short cut federal policy by limiting the approach of propagation to fish host techniques only.



Propagation Techniques

- **Difficulties with In Vitro Transformation**

  - Glochidia readiness (viability)
  - Media contamination
  - External incubation factors (CO_2, pH, ^oC)
  - Transformation timing (removal from media)



In Vitro Propagation Results

- **Nearly 20 species have been successfully propagated using *in vitro* culture media techniques:**

• *A. plicata*	• *L. cardium*	• *P. grandis*
• *E. angustata*	• *L. siliquoidea*	•*P. cordatum*
• *E. complanata*	• *L. fasciola*	• *P.cataracta*
• *E. crassidens*	• *L. teres*	• *U. imbecillis*
• *F. ebena*	• *L. recta*	• *V. iris*
• *F. flava*	• *M. gigantia*	• *V. lienosa*

- **Propagation techniques (*in vitro*) have generated viable juveniles for toxicity testing, *in situ* monitoring, and reintroduction efforts**.

Success of *in vitro* cultured juveniles

- Growth- newly-transformed juvenile lengths - 320 µm

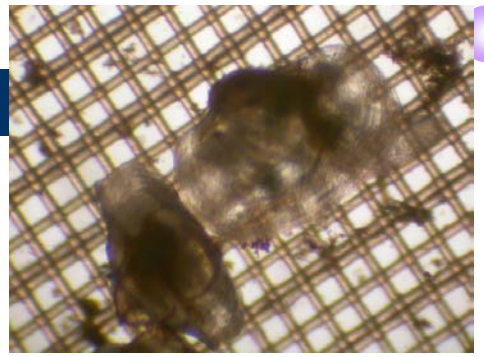
  simple static water system - 93 day growth

  - 3,257 µm in sediment from the Conasaugo River, TN;
  - 3,718 µm in sediment from Lake Monticello, SC;
  - 3,992 µm in sediment from the Telico River, TN.

- Survival

  - 60%, 53%, and 53%, respectively.
  - (On day 87, the survival of those in Lake Monticello sediment was 76% indicating a stressful change in just a few days, probably feeding needs! Hudson and McKissick (1999)

Successfully Used in Toxicity Testing for over 10 Years



Juvenile (*vivo* and *vitro*) toxicity testing

- Wade et al. 1989
- Jacobson 1990
- Keller and Zam 1991
- Lasee 1991
- Jacobson et al. 1993
- Johson et al. 1993
- Farris et al. 1995
- Warren 1996
- Scheller 1997
- Keller and Ruessler 1997
- Keller et al. 1998
- Clem 1998
- Hudson et al. 2003

More research needed!

- Are in vitro juveniles of equal physiological health as in vivo juveniles?